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Oxidative Stress and Inflammation in Brain Aging: Nutritional Considerations

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Aging can be defined as the condition where stressors are not counteracted by protective functions, leading to a dysregulation in development. These changes can be translated into decrements in neuronal functioning accompanied by behavioral declines, such as decreases in motor and cognitive performance, in both humans and animals. When coupled with genetic alterations, the ultimate expression of these changes is seen in diseases such as Alzheimer disease (AD). This association will be discussed in the last section of this chapter. In this review we will describe motor and cognitive deficits in behavior due to aging, and show how these deficits are related to increased vulnerability to oxidative stress, inflammation or signaling. Importantly, using muscarinic receptors as examples, we will also try to show that the sensitivity to these insults may be differentially expressed among neurotransmitter receptor subtypes.

KEY WORDS: Aging; behavior; inflammation; nutrition; oxidative stress.

BEHAVIORAL CHANGES IN AGING

Many behavioral changes that include both motor (1,2) and cognitive (3) declines during "normal" aging have been shown in numerous experiments. These studies have revealed that the deficits in motor function include decreases in balance, muscle strength, and coordination (1). Motor deficits are thought to be the result of either alterations in the striatal dopamine (DA) system, as the striatum shows marked neurodegenerative changes with age (4), or in the cerebellum which also shows age-related alterations (5,6).

Accompanying the alterations in motor behavior are deficits in cognitive performance. Observations of these changes have been made for a number of years on tasks involving spatial learning and memory (3,7–10). Memory alterations appear to occur primarily in secondary memory systems that involve the storing of new information (4,11). Research has suggested that various brain regions are involved in aspects of this storage including: a) the hippocampus which mediates place learning, b) the prefrontal cortex which is critical to acquiring procedural knowledge, and c) the dorsomedial striatum which mediates egocentric response and cue learning (12–15). While the mechanisms involved in both motor and cognitive deficits during aging remain to be discerned, it is clear that oxidative stress (16) and inflammation (17,18) are involved.

Oxidative Stress

Numerous studies have reported indications of increased oxidative stress (OS) in brain aging including increases in bcl-2 (19) and membrane lipid

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peroxidation (22) along with reductions in redox active iron (20,21). Additionally there is significant lipofuscin accumulation (20) and alterations in membrane lipids (23).

OS vulnerability in aging also may be the result of additional factors including microvasculature changes and increases in oxidized proteins and lipids (24), as well as alterations in: a) membrane microenvironment and structure (25,26); b) calcium buffering ability; and c) the vulnerability of neurotransmitter receptors to oxidative stress (see below). Additional "vulnerability factors" include critical declines in endogenous antioxidant protection, involving alterations in the ratio of oxidized to total glutathione (27), and reduced glutamine synthetase (28). Findings suggest that age-related changes in neuronal lipid raft molecular structure and physical properties (e.g., increased rigidity) may increase vulnerability to oxidative stress and inflammation (25,26). Recent studies have suggested an involvement of lipid rafts with oxidative stress sensitivity (29). Taken together, these findings indicate that there are increases in OS in aging, that the CNS may be particularly vulnerable to these increases (see 25,30 for review), and the efficacy of antioxidants may be reduced in aging. As discussed below, it is also important to note along with increased sensitivity to OS, there may be associated increases in inflammation, (25,26).

Inflammation

Evidence also suggests that in addition to oxidative stress, CNS inflammatory events may have an important role in affecting neuronal and behavioral deficits in aging (31). As an example, increased glial fibrillary acid protein expression is observed by middle age (32), and in the elderly this increase even occurs in the absence of a defined stimulus (33). Increases in TNFα have also been reported as a function of age (34), as well as associated inhibition of glia (35). Similarly, research in both aged mice and humans (34,36,37) has found increases in both TNFα and IL-6. Up-regulation of C-reactive protein, an important marker of inflammation, may be an important factor in biologic aging (38). All of these changes appear to be accompanied by up-regulations in downstream indicators of inflammation (e.g. complement C1q) in microarray studies (39).

Just as has been observed with oxidative stress, increased sensitivity to inflammation is also seen in aging. In this respect Manev and Uz (40) have shown an enhanced sensitivity in aged rats to kainate-induced excitotoxic brain injuries associated with increased 5-lipoxygenase (5-LOX) expression in limbic structures. Studies indicated this inflammatory marker (5-LOX) is expressed in CNS neurons and may be involved in neurodegenerative processes. Findings indicate that 5-LOX may exert its actions through tyrosine kinase receptors and cytoskeletal proteins (reviewed in 41), and it is known that 5-LOX gene expression and activity are increased in aging. Cyclooxygenase and 5-lipoxygenase inhibitors can protect against neurotoxicity (42) presumably by reducing 5 Lox expression.

Additionally, studies indicate that the expression of one form of COX, 2, appears to be associated with amyloid beta deposition in the hippocampus 43,44) Moreover, research has suggested that inflammatory prostaglandins (PG) such as PGE increase in the hippocampus, as well as other areas in aging (45). Since the PG synthesis pathway appears to be a major source of reactive oxygen species (ROS) in the brain (46), and in other organ systems, these findings indicate that inflammation may be accompanied by and even generate its "evil twin", OS in producing the deleterious effects of aging. Thus, such factors as cytokines, cycloogenases, prostaglandins, etc. may act as extracellular signals in generating additional ROS that are associated with decrements in neuronal function or glial neuronal interactions (47–51) and ultimately the deficits in behavior that have been observed in aging.

If this is the case then it should be possible to produce deficits in cognitive and motor function similar to those seen in aging by using procedures that enhance inflammation and/or oxidative stress. Such experiments involving the use of heavy particle irradiation, central injections of lipopolysaccharide, or kainic acid will be discussed in the next section.

OXIDATIVE AND INFLAMMATORY INDUCED COGNITIVE AND MOTOR DEFICITS

Findings indicate that young animals exposed to oxidative or inflammatory stressors exhibit similar neuronal and behavioral changes to those seen in aging. For example, studies have shown that exposing young rats to particles of high energy and charge (HZE particles) or heavy particle irradiation, which acts to increase oxidative and/or inflammatory stressors, produces behavioral deficits paralleling those observed in aging (52–54). HZE particles

(specifically 600 MeV or 1 GeV ^{56}Fe) also disrupt the functioning of the dopaminergic system and dopamine-mediated behaviors, such as motor behavior (55), spatial learning and memory behavior (54), and amphetamine-induced conditioned taste aversion (56).

These findings are supported by additional studies in which a normobaric hyperoxia environment of 100% oxygen (O_2) at 760 mm Hg (sea level pressure) was used in young rats to assess motor function. Following 48 h of 100% O_2, rats showed deficits in performance on strength and balance tests (16), as well as cerebellar ß-adrenergic and striatal muscarinic receptor functioning (57). Again, these effects are similar to those seen in aging.

An additional treatment involving the induction of OS by reducing the levels of the endogenous antioxidant glutathione with buthionine sulfoximine (BSO), followed by central DA administration to create conditions similar to those seen in aging, also produced decrements in both cognitive and motor performance. The results showed that BSO given prior to DA administration selectively impaired psychomotor (58) and cognitive performance (59). Neither BSO alone nor DA alone had detrimental effects on behavior.

Similar changes in behavior are seen with experimentally induced increases in inflammatory mediators (e.g. cytokines) known to be involved in the activation of glia cells, perivascular/parenchymal macrophages, and increased mobilization and infiltration of peripheral inflammatory cells into the brain (17). It appears that central administration of lipopolysaccharide (LPS) produces increases in several markers of inflammation and results in degeneration of hippocampal pyramidal neurons, as well as impairments in working memory (60–62). In fact, chronic (28–37 days) infusion of LPS into the ventricle of young rats can reproduce many of the behavioral, inflammatory, neurochemical, and neuropathological deficits seen in the brains of AD patients in regions such as the cingulate cortex, which are also affected in AD (17,18,60–62). Changes in the inflammatory and behavioral markers were accompanied by increases in: a) the number of activated astrocytes, b) the quantity and density of activated microglia, particularly within the hippocampus, cingulate cortex, and basal forebrain, and c) the levels of cytokines, and degeneration of hippocampal pyramidal neurons.

A more recent study (63) has shown that kainic acid induces increases in hippocampal Il-1β and increases in OX-6 activation accompanied by declines in cognitive performance on a Morris water maze, further suggesting an important role for inflammatory processes in motor and cognitive deficits in aging.

MUSCARINIC RECEPTORS

One other important contributor to increased OS in aging is the decline in calcium buffering (64,65). Such losses can have a profound effect on the functioning and viability of the cell (66–68). In effect, a vicious circle is produced with OS inducing decrements in calcium buffering (69), which lead to further increases in OS (70), resulting in possible decrements in motor and memory function in senescent rats (see above).

One area where the changes in calcium buffering may be especially salient is in muscarinic receptors. Deficits in the sensitivity of striatal muscarinic receptors as assessed via oxotremorine-enhancement of K^+-evoked DA release and carbachol-stimulated GTPase activity were observed in aging and AD (see 26 for review), as well as in irradiated rats (53). Given that MAChRs are found in memory control areas (71) and in the vasculature (72,73), changes in sensitivity may be important to OS.

More recent evidence suggests that muscarinic receptor activation provides protection against possible OS and subsequent apoptosis (70). Additionally, Fawcett and colleagues (74) showed that a low molecular weight endogenous inhibitor from AD brains decreases oxotremorine-M binding (75). Finally, MAChRs are involved in various aspects of both neuronal (76) and vascular functioning (72).

It also appears that the oxidative stress sensitivity may not be uniform among the various MAChR subtypes. Findings have indicated that COS-7 cells transfected with one of the five MAChRs and exposed to DA (69) or amyloid beta (Aβ) (77) showed differences in OS sensitivity expressed as a function of Ca^{2+} buffering, assessed by examining the ability of the cell to extrude or sequester Ca^{2+} following oxotremorine-induced depolarization. COS-7 cells transfected with M1, M2, or M4AChR showed greater sensitivity to DA or Aβ than those transfected with M3 or M5AChR. These findings may begin to explain previous results showing significant differences in the rates of aging among various brain regions. Areas such as the hippocampus (78,79), cerebellum (79,80) and striatum (81,82) show profound alterations in aging in such factors as morphology, electrophysiology and receptor sensitivity.

In this regard Hersch and colleagues (83) have shown that M1 receptor protein is expressed in 78%

of the neurons of the striatum, while M2 receptors may be the predominant muscarinic receptor in the striatum, and M4 receptors were localized to 44% of striatal cells. In addition, a wide distribution in these receptor subtypes has been seen in the dentate (71), and M2 and M4 MAChR receptor proteins were found in high concentrations in the fimbria-fornix. It also appears that M1 receptors were found to be enriched in the forebrain in primates (84).

Structurally, it is not clear how the variations in oxidative stress sensitivity are imparted to the various MAChR subtypes. However, recent evidence indicates that this may occur through the i3 loop. Indirect evidence for the importance of i3 loop in oxidative stress sensitivity is provided by studies (85–87) showing that G protein ∃ interacts with the i3 loop of the MAChR, is inhibited by the G protein ∀ subunit, and is necessary for phosphorylation and subsequent signal transduction. Studies have shown reductions in muscarinic signaling that involve decrements in muscarinic receptor-G protein coupling/uncoupling in aging and AD (81).

More direct findings show the possibility that the i3 loop is involved in the regulation of oxidative stress (88). Deletions of the entire i3 loop increased DA sensitivity (a lower % of cells showing recovery following depolarization) in both the M1 and M3 subtypes. Chimerics of M1 where the i3 loop of the M3AChR was switched with the i3 loop of the M1AChR (M1M3i3) showed that the DA sensitivity was reduced (% cells showing increases in calcium clearance) following depolarization. In the M3 chimerics containing M1i3 (M3M1i3), the i3 loop offered no protection against DA-induced decrements in calcium buffering. Results suggest that the longer i3 loop of the M3AChR may be decreasing OS sensitivity and the possible targeting of antioxidants to specific receptor sites that impart oxidative stress sensitivity (88).

Interestingly, it also appears from our previous findings (69,77) that the MAChRs which conferred the most OS sensitivity to the transfected COS-7 cells (i.e., M1, M2, M4) had fewer base pairs in their i3 loops than the M3 or M5 MAChRs, suggesting that base pair length might be involved in regulation of OS sensitivity.

THE EFFECTS OF FRUIT AND VEGETABLE SUPPLEMENTATION ON BEHAVIORAL AND NEURONAL DEFICITS IN AGING

It appears from the above discussion that there are increases in sensitivity to oxidative stress and inflammation in the aged brain that could lead to motor and cognitive deficits. These vulnerability increases, when combined with: abnormal: APP processing, tau phosphorylation, and presenilin 1, could lead to alterations in brain morphology and cognitive diseases such as AD. Thus, the problem becomes reducing the vulnerability of the brain to oxidative stress and inflammation and preventing or reversing subsequent deficits in behavior. One approach to accomplish this task would be to utilize nutrition.

While there are numerous studies suggesting that various antioxidant supplements can be effective in this regard (see 89 for review), our research suggests that the combinations of antioxidant/anti-inflammatory polyphenolics found in fruits and vegetables may show efficacy in aging. All plants, including fruit or vegetable bearing plants, synthesize a vast array of chemical compounds that are not necessarily involved in the plant's metabolism. These secondary compounds' instead serve a variety of functions that enhance the plant's survivability. These compounds may be responsible for the putative multitude of beneficial effects of fruits and vegetables on health-related issues, two of the most important of which may be their antioxidant and anti-inflammatory properties.

The anthocyanins are among the plant polyphenols that have potent antioxidant and anti-inflammatory activities. These are natural pigments responsible for the orange, red and blue colors of fruits, flowers, vegetables and other storage tissues in plants (90–92). Anthocyanins have been reported to affect many of the parameters discussed above by inhibiting lipid peroxidation and the activity of cycloxygenase-1 and -2 (COX) enzymes (93,94).

Anthocyanins are a subset of a larger class of polyphenols known as flavonoids. Flavonoids have been reported to inhibit lipid peroxidation in several biological systems including mitochondria and microsomes (95,96), as well as erythrocytes (97,98) and liver (99), and are potent inhibitors of 5-LOX (100).

The antioxidant effects of flavonoids also appear to involve transcriptional up-regulation of antioxidant enzymes related to glutathione synthesis and/or glutathione. For example, the enzymes for glutathione (reviewed in 101,102) or heme oxygenase (103) synthesis are dependent on ERK1/2. It also appears that flavonoids regulating ERK1/2 may influence iNOS activity. Thus there is a great deal of evidence to suggest that a possible link exists between the antioxidant activity of flavonoids and their putative MAP-kinase altering activity.

Since MAPK's are involved in numerous biological activities, the findings that flavonoids may influence such signaling suggests that their potential benefits may involve properties other than those involving antioxidant or anti-inflammatory effects. As examples, delphinidin inhibits endothelial cell proliferation and cell cycle progression by ERK 1/2 activation (104), while grape seed proanthocyanidin can reduce ischemia reperfusion-induced activation of JNK-1 and C-Jun, and reduce cardiomyocyte apoptosis (105). Additional research indicates that phytochemicals can regulate MAP kinase and other signaling pathways at the level of transcription (106).

Given the numerous studies showing the involvement of ERK in diverse forms of memory, such as: contextual fear conditioning (107); long-term potentiation (108); striatal-dependent learning and memory (109); hippocampal-dependent spatial memory (110); and inhibitory avoidance (111), these findings suggest that the putative signaling modifying properties of flavonoids may prove to be invaluable in altering the neuronal and behavioral effects of aging.

Animal model studies have shown that long-term (from 6 to 15 months of age; F344 rats) feeding with a supplemented AIN-93 diet [strawberry extract or spinach extract (1–2% of the diet) or vitamin E (500 IU)], retarded age-related decrements in cognitive or neuronal function. Results indicated that the supplemented diets could prevent the onset of age-related deficits in several indices (e.g., cognitive behavior and Morris water maze performance) (112).

An additional experiment has found (113) that dietary supplementation (for 8 weeks) with spinach, strawberry or blueberry (BB) extracts in an AIN-93 diet was effective in reversing age-related deficits in neuronal and behavioral (cognitive, Morris water maze performance) function in aged (19 mo) F344 rats. Only the BB supplemented group exhibited improved performance on tests of motor function that assessed balance and coordination, (e.g., rod walking and the accelerating rotarod), while none of the other supplemented groups differed from control on these tasks.

Unlike the results seen with respect to motor behavior, our study (113) showed that the animals in all supplemented groups (relative to controls) showed improved working memory (short-term memory), as measured by performance in the Morris water maze, suggesting less selectivity among fruits and vegetables with respect to cognition than that seen with motor behavior. This may be the result of brain region selectivity of the polyphenolic compounds from the various fruits and vegetables.

Extending these findings, assessments were made of the effectiveness of BB and other high antioxidant fruit extracts (boysenberry; cranberry; black currant; strawberry; dried plums; and grape) on the toxic effects of Aβ 25–35 and DA on calcium buffering following oxotremorine (750 μM)-induced depolarization in M_1AChR-transfected COS-7 cells, and on cell viability following DA (4 h) exposure. All of the extracts produced some protection against Aβ or DA exposure (114). This is an important finding as the Aβ peptide promotes calcium influx and disrupts the calcium homeostasis that also occurs in aging (80,115). The ability of the various fruit extracts to prevent these disruptions suggests that they may be useful in preventing or perhaps reversing the deleterious effects of AD.

In this regard, a recent study (116), carried out in APP/PS1 transgenic mice given BB supplementation (as in 113) beginning at 4 months of age and continued until they were 12 months of age, exhibited Y-maze performance that was similar to those seen in non-transgenic mice and significantly greater than that seen in the non-supplemented transgenic animals. Interestingly, there was a dichotomy between the plaque burden and behavior in the BB-supplemented transgenic mice. No differences between the supplemented and non-supplemented APP/PS1 mice in the number of plaques were observed, even though behavioral declines were prevented in the BB-supplemented animals (116).

Subsequent analyses indicated that there was enhanced signaling present in the BB-supplemented transgenic mice that acted to prevent or circumvent any putative deleterious effects of the amyloid plaques on behavior (116). The evidence for this possibility is provided by data showing that the BB-supplemented APP/PS1 mice exhibited greater levels of hippocampal extracellular signal regulated kinase (ERK), as well as striatal and hippocampal protein kinase Cα (PKC) than that seen in the transgenic mice maintained on the control diet (116). Enhancement was also seen in the BB-supplemented group in the sensitivity of muscarinic receptors (i.e., increasing striatal, carbachol-stimulated GTPase activity) which have been found to be associated with learning and memory in numerous studies.

These findings, combined with additional research showing that BB supplementation, in addition to altering ERK activity, may also increase hippocampal neurogenesis (117), suggests that at

least part of the efficacy of the BB supplementation may be to enhance neuronal function in areas of the brain affected by aging or disease. This would allow more effective intra- and inter-area communication and ultimately facilitate both cognitive and motor function. Thus, nutrition may prove to be a valuable asset in ''quenching the fires'' of inflammation and OS in aging and perhaps AD. Since oxidative stress is an early change in aging that is superimposed upon a stress vulnerable aging brain, early nutritional intervention may prevent or delay the onset of this disease

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